

SPECIFICITY OF SYSTEMICALLY RELEASED COTTON VOLATILES AS ATTRACTANTS FOR SPECIALIST AND GENERALIST PARASITIC WASPS

URSULA S. R. RÖSE,^{1,2} W. JOE LEWIS,³
and JAMES H. TUMLINSON^{1,*}

¹Center for Medical, Agricultural and Veterinary Entomology
Agricultural Research Service, U.S. Department of Agriculture
Gainesville, Florida 32608

³Insect Biology and Population Management Research Laboratory
Agricultural Research Service, U.S. Department of Agriculture
Tifton, Georgia 31793

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Abstract—Cotton plants under herbivore attack release volatile semiochemicals that attract natural enemies of the herbivores to the damaged plant. The volatiles released in response to herbivory are not only released from the damaged leaves but from the entire cotton plant. We found that cotton plants that released myrcene, (Z)-3-hexenyl acetate, (E)- β -ocimene, linalool, (E)-4,8-dimethyl-1,3,7-nonatriene, (E)- β -farnesene, and (E, E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene systemically from undamaged leaves of caterpillar damaged plants were attractive to the generalist parasitoid *Cotesia marginiventris* and the specialist parasitoid *Microplitis croceipes*. Plants from which the caterpillar damaged leaves were removed and that released those compounds systemically were significantly preferred over undamaged control plants in two-choice experiments in a flight tunnel. Artificially damaged cotton plants that released green leafy volatiles and constitutive terpenoids were less attractive for *M. croceipes* and *C. marginiventris*. Only *C. marginiventris* preferred artificially damaged plants over undamaged control plants, whereas *M. croceipes* showed no preference. The apparent lack of specificity of systemically released compounds in response to different herbivores feeding on the lower leaves is discussed.

Key Words—*Gossypium hirsutum*, cotton, parasitoids, *Microplitis croceipes*, *Cotesia marginiventris*, plant-insect interactions, volatile semiochemicals, systemic induction, plant defense, host-searching behavior, generalist, specialist.

*To whom correspondence should be addressed.

²Present address: Max-Planck Institute für Chemische Ökologie, Sophienstrasse 10, 07743 Jena, Germany.

INTRODUCTION

Several plant species are known to release volatile compounds when under herbivore attack (Dicke et al., 1990a; Turlings et al., 1990; McCall et al., 1994; Loughrin et al., 1994). This herbivore-induced release of volatiles benefits the plant by attracting natural enemies of the herbivores that feed on its foliage and benefits parasitoids and predators by guiding them to potential hosts or prey on the plant (Dicke and Sabelis, 1988; Dicke et al., 1990b; Turlings et al., 1991a,b; Takabayashi et al., 1991; McCall et al., 1993). The volatile compounds released from damaged plants can be divided into constitutive compounds and inducible compounds. Constitutive compounds are continuously present in the plant and are released from damaged leaves immediately after the beginning of feeding damage or even after the plant is only artificially damaged with a razor blade to mimic the mechanical part of feeding damage (Turlings et al., 1990; R  se et al., 1996). Early stages of plant damage are characterized by the release of "green leafy" volatiles such as (Z)-3-hexenal, (Z)-3-hexenol, (Z)-3-hexenyl acetate, and the constitutive compounds that are plant specific (McCall et al., 1994; Loughrin et al., 1994; Turlings et al., 1995). Constitutive compounds in cotton are monoterpenes and sesquiterpenes (Loughrin et al., 1994) that are stored in laticiferous glands (Elzen et al., 1985). After several hours of herbivore damage or on the next day, the plants start to release additional compounds that appear to be specifically released in response to herbivore damage. Their release from damaged leaves is not elicited in significant amounts by artificial damage alone (Turlings et al. 1990; Par   and Tumlinson, 1997a; R  se et al., unpublished data). These herbivore-inducible compounds in cotton are acyclic terpenoids [i.e., (E)-  -ocimene, (E)-  -farnesene, (E,E)-  -farnesene, linalool, (E)-4,8-dimethyl-1,3,7-nonatriene, (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene] and (Z)-3-hexenyl acetate, indole, isomeric hexenyl butyrates, and 2-methylbutyrates (McCall et al., 1994; Loughrin et al., 1994).

Several of the inducible compounds in cotton are not only released at the damaged site, but are released systemically throughout the entire plant (R  se et al., 1996). After feeding damage of beet armyworm larvae *Spodoptera exigua* H  bner (Lepidoptera: Noctuidae) on the lower leaves of a cotton plant for several days, the upper undamaged leaves of the same plant released (Z)-3-hexenyl acetate, (E)-  -ocimene, linalool, (E)-4,8-dimethyl-1,3,7-nonatriene, (E)-  -farnesene, (E,E)-  -farnesene, and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (R  se et al., 1996).

In cotton, the release of inducible terpenes [(E)-  -ocimene, (E)-4,8-dimethyl-1,3,7-nonatriene, (E)-  -farnesene, (E,E)-  -farnesene] from the entire damaged plant follows a diurnal cycle, with peak emission of volatiles in the early afternoon (Loughrin et al., 1994). The timing of the plant signal coincides

with the time of active foraging of parasitic wasps (Snow and Burton, 1967; Lewis et al., 1972; Turlings et al., 1995), and several parasitoids and predators are known to exploit herbivore-induced compounds to lead them to those plants where they are likely to encounter a host or prey (Dicke and Sabelis, 1988; Turlings et al., 1990; Dicke and Dijkman, 1992; McCall et al., 1993).

The systemic release of inducible compounds in cotton plants allowed us to separate the attraction of parasitoids to inducible compounds from the attraction to constitutive compounds in behavioral bioassays in the flight tunnel. We conducted experiments with the generalist parasitoid *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) and the specialist parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) to observe whether the systemic release of inducible compounds would increase the attractiveness of the cotton plant for the parasitoids. *C. marginiventris* can parasitize larvae of the noctuid species *S. exigua*, *Spodoptera frugiperda* J. E. Smith, and *Helicoverpa* and *Heliothis* spp., whereas *M. croceipes* is an endoparasitoid of *Heliothis* and *Helicoverpa* spp. but cannot develop in *S. exigua* larvae (Blumberg and Ferkovich, 1994). *S. exigua* larvae were used to induce the systemic volatile release for our flight-tunnel experiments. To confirm that cotton plants that were used for flight-tunnel experiments released inducible compounds, their volatiles were collected immediately prior to the flight-tunnel experiments. In addition, we determined the specificity of the systemic plant signal to the attacking herbivore species. In a second set of two-choice flight tunnel experiments, we compared the attractiveness to the parasitoids of artificially damaged cotton plants that released constitutive volatile compounds and undamaged control plants.

METHODS AND MATERIALS

Plants. Approximately 6-week-old cotton plants, *Gossypium hirsutum* L. (Malvaceae), var. Deltapine acala 90, with six fully developed leaves in addition to the two cotyledons, were used in all experiments. Cotton was grown in a greenhouse in a mixture of compost, peat moss, and vermiculite (Metro-mix 300, Scotts-Sierra Horticultural Company, Marysville, Ohio) with natural light, under Florida summer conditions (14L:10D light cycle, $85 \pm 10\%$ relative humidity, and $35 \pm 10^\circ\text{C}$). Each plant was grown from seed planted in a 16-cm-diameter pot and fertilized once at time of planting with a three to four-month formulation of Osmocote 14-14-14 (N-P-K) controlled-release fertilizer (Scotts-Sierra Horticultural Products).

Lepidoptera Larvae. Beet armyworm larvae (BAW) and corn earworm (CEW) *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) larvae were obtained from the USDA rearing facilities in Gainesville, Florida. Larvae were reared

on an artificial diet based on pinto beans according to the method of King and Leppla (1984). To encourage immediate feeding of larvae after being caged on leaves, third instars were starved for 24 hr prior to the experiments.

Parasitoids. The specialist larval endoparasitoid *M. croceipes* and the generalist larval endoparasitoid *C. marginiventris* were reared from cocoons obtained from colonies maintained at the US Department of Agriculture-Agricultural Research Service, Insect Biology and Population Management Research Laboratory, Tifton, Georgia. *M. croceipes* were reared on larvae of *H. zea* fed on CSM diet (Blended Food Product, Child Food Supplement, Formula No. 2) as previously described (Burton, 1970; Lewis and Burton, 1970). *C. marginiventris* of the 1985 Mississippi strain were reared on larvae of *S. frugiperda* fed on soybean diet as described by Lewis and Burton (1970). Female and male parasitoids were kept together in screen cages (25 × 25 × 25 cm) in the laboratory to allow mating and were fed with honey and water after emergence. Females were separated in cages according to their date of emergence. Mated females used for flight tunnel experiments were 3–4 days old and kept in the laboratory at 24 ± 2°C, 55 ± 5% relative humidity, and on a 14L:10D light cycle.

Caterpillar Feeding on Lower Cotton Leaves. To determine the specificity of the systemically released chemicals after herbivore attack, volatiles were collected from the undamaged upper leaves after feeding damage of CEW on the lower leaves and compared to compounds systemically released after feeding damage of BAW larvae. For this, a total of either four BAW or four CEW were placed on the two lower leaves of separate plants. On each leaf, two BAW or two CEW were enclosed in a cage that consisted of two modified halves of a Petri dish as previously described (Röse et al., 1996). BAW or CEW larvae were placed on the lower leaves on day 1 at 11:00 hr and were replaced with new, starved larvae every day for four days. On day 4 at 11:00 hr, volatiles were collected from the undamaged upper leaves of the BAW-damaged plant (SYST-BAW) and from the undamaged upper leaves of the CEW-damaged plant (SYST-CEW) for 1 hr as previously described (Röse et al., 1996). Volatiles were also collected from upper leaves of undamaged control plants (SYST-CTRL) in the same way.

Artificially Damaged Cotton Plants. For the collection of volatiles from artificially damaged cotton plants (ART), the third and fourth leaves (counted starting from the youngest leaves) were damaged in the center with a garlic press (Ekco Housewares, Franklin Park, Illinois), inflicting 32 holes of approximately 1 mm² each over an area of 15 × 35 mm/leaf. Volatile collections from ART plants that included the damaged leaves were conducted from 12:00 to 15:00 hr on different plants from the ones used for flight-tunnel experiments. Separate plants were used to avoid a decrease in volatiles passively released from the damaged leaf. Volatiles were also collected from undamaged control plants (CTRL) in the same way from 11:00 to 15:00 hr.

Volatile Collection. To collect volatiles from SYST-BAW, SYST-CEW, and SYST-CTRL, the upper four leaves of each plant were enclosed in separate volatile collection chambers as previously described (Röse et al., 1996). These were part of an automated volatile collection system as previously described (Heath and Manukian, 1992, 1994; Manukian and Heath, 1993). This system allows for the collection of volatiles from the upper portion of the plants while completely isolating the lower section of the plant where caterpillars are feeding in the systemic treatment.

Volatiles emitted from the upper portion of the plant enclosed within the glass chamber were swept downward by the incoming pure laminar airflow at a rate of 5 liters/min. They were sampled at the bottom of the chamber by pulling air at a rate of 1 liter/min through volatile collection traps (Super-Q, 50 mg) (Röse et al., 1996) with a controlled vacuum source attached to each volatile collector trap from the automated volatile collection system. Thus, 20% of the air passed through the collector traps during the 1-hr collection period. The remaining 80% excess air escaped through a small opening around the stem of the plant at the bottom of the collection system. This positive pressure venting provided a barrier against all ambient air and prevented volatiles from the lower, damaged part of the plant from entering into the collection chamber containing the upper, undamaged part of the plant. Details of the release of volatiles from the undamaged parts of a caterpillar damaged plant and a control plant over four days were reported by Röse et al. (1996).

Volatiles from ART plants were collected with the same collection system from 12:00 to 15:00 hr, by enclosing all cotton leaves in the collection chamber, including the artificially damaged ones, while the pot remained outside the system. These plants were artificially damaged on the third and fourth leaf and immediately placed in the chamber for collection of 20% of the volatiles. Volatiles were collected at the same time from CTRL plants.

Analysis of Volatiles. Volatiles were extracted from the collector traps by washing with 170 μ l methylene chloride (capillary GC/GC-MS solvent, Burdick & Jackson, Muskegon, Michigan). Internal standards (600 ng each of *n*-octane and nonyl acetate in 60 μ l methylene chloride) were added to the extract. Of each collection sample, 1 μ l was analyzed on a Hewlett-Packard gas chromatograph (model 5890 II plus). Samples were injected by a Hewlett-Packard auto injector (model 6890) in split-splitless mode. The GC was equipped with a flame ionization detector. Data collection, storage, and subsequent analysis was performed on a Perkin Elmer chromatographic data system. Helium at a linear flow velocity of 20 cm/sec was used as a carrier gas. All samples were analyzed on a fused silica capillary column (Quadrex Corporation, New Haven, Connecticut), 50 m \times 0.25 mm ID, with a 0.25- μ m-thick film of bonded methyl silicone (007). The temperature of the column oven was maintained at 40°C for 3 min, and then programmed at 5°C/min to 220°C, which was maintained for

10 min. The injector temperature was set at 220°C, the detector temperature at 260°C.

To identify compounds, volatiles were analyzed by GC-mass spectroscopy (GC-MS) with a Finnigan ITS-40 Magnum (ion-trap) mass spectrometer operated in electron impact and chemical ionization modes. For GC-MS, a fused silica capillary column was used with helium as a carrier gas, and for chemical ionization, isobutane was used as reagent gas. Constituents of the plant volatile emission were identified by comparison of mass spectra with spectra in the Environmental Protection Agency-National Institutes of Health data base, the Environmental Protection Agency-National Institute of Standards and Technology data base, and spectra obtained from authentic compounds. GC retention times of plant volatiles also were compared with GC retention times of authentic compounds on the methyl silicone column.

Flight Tunnel. All free flight experiments with *M. croceipes* and *C. marginiventris* were carried out in a Plexiglas flight tunnel 60 × 60 cm in cross section and 240 cm long, as previously described (Eller et al., 1988; Turlings et al., 1991a). The windspeed was adjusted to 0.2 m/sec. Air was exhausted outside the building after being drawn through the tunnel. A pattern of 20-cm-wide black and white stripes under the tunnel was used to provide flying insects with a visual reference. Four 90-W krypton lights illuminated the flight tunnel with approximately 800 lux from above (measured with an Extech Instruments light meter, Davis Instruments, Baltimore, Maryland). A temperature of $27 \pm 1^\circ\text{C}$, and $75 \pm 5\%$ relative humidity was maintained in the tunnel during the experiments. Wasps used for flight-tunnel experiments were transferred to the flight tunnel room 3 hr prior to the experiment to adjust to the room conditions and were used 4–6 hr into the photophase. All wasps were used only once in an experiment.

Preflight Experience. To increase the responsiveness of the parasitoids, females of *M. croceipes* were first allowed to antennate and parasitize a third instar CEW fed on pinto bean diet and were immediately afterwards (within 3 min) released in the flight tunnel. Females of *C. marginiventris* were first allowed to antennate and parasitize a third-instar BAW fed on pinto bean diet. Wasps that were injured or came in contact with larval oral secretion during oviposition were discarded.

Flight-Tunnel Experiments. Only cotton plants that were fed upon by BAW and for which a systemic volatile release was confirmed by collection and analysis of volatiles were used for flight tunnel experiments (SYST-BAW). After the volatile collection, at 12:00 hr, SYST-BAW plants were prepared for the flight tunnel by removing caterpillars from the lower leaves and cutting the damaged leaves off to remove any volatile or visual cues resulting from the feeding site itself. The cut leaf stems of the plant were wrapped in Teflon tape to minimize the release of volatiles from the cut. SYST-CTRL plants were

treated in the same way, and two lower leaves that were matched for size and position with the SYST-BAW plants were removed. The cut leaf stems of the plant were wrapped in Teflon tape like SYST-BAW. Both the SYST-BAW and SYST-CTRL plant, with the lower leaves removed, were placed in the flight tunnel for two-choice flight experiments.

To compare ART plants and CTRL plants, initially only the third leaf (counted starting from the youngest leaves) of ART plants was damaged prior to placing the plant in the flight tunnel. After 1 hr of experiments, or after 12 wasps were flown in the flight tunnel, an additional leaf at the fourth position was damaged to ensure continuous release of volatiles. An undamaged cotton plant matched for size and leaf number was used as a control plant for two-choice experiments in the flight tunnel (CTRL).

No-Choice Flight Tunnel Experiments. To establish a baseline response of *C. marginiventris* and *M. croceipes* to undamaged cotton plants, we observed the number of completed flights to CTRL plants in a no-choice situation.

Two-Choice Flight Tunnel Experiments. All two-choice experiments compared the flight response to a treated plant and an undamaged control plant. The first set of experiments investigated the attraction of *C. marginiventris* and *M. croceipes* to herbivore inducible volatiles systemically released from SYST-BAW plants compared to SYST-CTRL plants. The second set compared responses to ART plants with responses to CTRL plants. These experiments were conducted to distinguish the responses of parasitoids to constitutive cotton volatiles from responses to inducible volatiles specifically released in response to herbivore damage. The position of the plants in the flight tunnel was switched routinely for each pair to avoid positional bias. Both plants were positioned equidistant from the parasitoid release point. Parasitoids were released individually 80 cm downwind of the odor source in a glass cylinder 25 cm above the tunnel floor. The glass cylinder ended in a curved funnel opening into a glass tube (details described by Turlings et al., 1991b) that was oriented parallel to the airflow. The odors released upwind could pass through the glass tube, but the tube prevented the insects from taking flight before experiencing the odors. In all bioassays, each parasitoid was given three chances to complete a flight by landing on a plant after a nonstop flight. After an incomplete flight, the parasitoid was returned to the release chamber to be released again. The choice of the parasitoid after a completed flight was recorded, as was the number of wasps that did not complete flights.

Statistical Analyses. The flight response of both parasitoid species was investigated on five separate days, with a total of 60 wasps tested for their attraction to the plants in each no-choice experiment (CTRL) and in each two-choice experiment (SYST-BAW vs. SYST-CTRL and ART vs. CTRL). Differences in the total numbers of wasps that completed a flight to a plant over all experiments and differences within each two-choice experiment in the total

number of wasps responding to each choice were analyzed by Fischer's exact test with the statistic program Systat (Systat Inc., Evanston, Illinois). The t test was used to determine differences in the amounts of volatiles released per plant from SYST-BAW and SYST-CEW leaves, for pairwise comparisons of those plants to ART and CTRL leaves, and for pairwise comparisons of SYST-CTRL and CTRL plants. Observed volatile amounts were summarized by the mean and corresponding standard error. Comparisons yielding $P \leq 0.05$ were considered to be statistically significant.

RESULTS

The total amount of volatiles released from the plants varied with the different treatments. While the totals released from both systemic treatments were similar (SYST-BAW: 12.58 $\mu\text{g/hr}$, SD = 6.8 $\mu\text{g/hr}$; SYST-CEW: 12.02 $\mu\text{g/hr}$, SD = 7.1 $\mu\text{g/hr}$) the totals released from ART (4.57 $\mu\text{g/hr}$, SD = 1.7 $\mu\text{g/hr}$) and CTRL (0.29 $\mu\text{g/hr}$, SD = 0.2 $\mu\text{g/hr}$) plants were both significantly ($P \leq 0.05$) smaller. Furthermore, the composition of the volatile blends released by the systemic treatments varied from the volatile composition released by ART and CTRL plants (Figure 1). Volatile compounds systemically released from SYST-BAW and SYST-CEW plants were mostly acyclic terpenoids [i.e., (*E*)- β -ocimene, (*E*)- β -farnesene, (*E,E*)- α -farnesene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene] and (*Z*)-3-hexenyl acetate. All these compounds were released in higher amounts from SYST-BAW and SYST-CEW plants compared to CTRL plants. In addition, small amounts of other inducible compounds [indole, (*Z*)-3-hexenyl butyrate] and small amounts of constitutive compounds were released.

Volatile compounds released from SYST-BAW and SYST-CEW showed no qualitatively and quantitatively significant differences (Figure 1A and B). Artificially damaged cotton leaves released green leafy volatiles [(*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate] and other constitutive compounds (α -pinene, β -pinene, myrcene, β -caryophyllene) (Figure 1C), whereas undamaged CTRL plants released only trace amounts of constitutive compounds and no measurable amounts of inducible compounds (Figure 1D). No differences were detected in the amounts of volatiles released from SYST-CTRL and CTRL leaves.

In the flight tunnel, both parasitoid species completed only 15–20% of flights to undamaged CTRL plants in a no-choice situation (Figure 2A and B; CTRL). These CTRL plants released only trace amounts of constitutive volatile compounds (Figure 1D). The total number of flights completed by the generalist *C. marginiventris* was significantly increased ($P \leq 0.032$) when an ART plant that released green leafy volatiles and constitutive compounds in larger amounts

(Figure 1C) was added to the flight tunnel for a two-choice experiment (Figure 2A; ART + CTRL). However, the addition of an ART plant to the CTRL plant did not increase the total number of flights completed by the specialist *M. croceipes* (Figure 2B; ART + CTRL). Parasitoids that were allowed to fly to SYST and SYST-CTRL plants showed a significant increase in the number of completed flights for *C. marginiventris* (Figure 2A; SYST + SYST - CTRL; $P \leq 0.01$) and *M. croceipes* (Figure 2B; SYST + SYST - CTRL; $P \leq 0.001$) when compared to ART and CTRL plants. SYST plants released high amounts of acyclic terpenoids (Figure 1A).

Besides differences between the parasitoid species in the total number of completed flights to the different plant treatments, we observed further differences in the choices the wasp species made between treated and control plants. The preference for SYST-BAW plants that released inducible volatile compounds over SYST-CTRL plants was highly significant for the generalist *C. marginiventris* (Figure 3A, $P \leq 0.001$) and the specialist *M. croceipes* (Figure 3C, $P \leq 0.001$). However, a difference between the two parasitoid species was observed when they were given a choice between ART and CTRL plants. The generalist *C. marginiventris* preferred ART plants that released constitutive volatile compounds over CTRL plants (Figure 3B; $P \leq 0.041$), whereas the specialist *M. croceipes* showed no preference for either plant (Figure 3D, $P \leq 1.0$).

DISCUSSION

The generalist parasitoid *C. marginiventris* and the specialist parasitoid *M. croceipes* both completed a small number of flights to undamaged cotton plants. Those undamaged plants released only trace amounts of volatiles and may be attractive to parasitoids by their color and shape alone. Wäckers and Lewis (1994) showed that *M. croceipes* uses visual as well as olfactory cues to locate a host site. The attraction to undamaged plants was displayed by the wasps after a preflight experience consisting of antennation and oviposition of a diet-fed host larva. This experience sensitizes and activates the host-searching behavior of a wasp and increases the number of completed flights compared to naive wasps (Röse et al., 1997). However, it does not provide the wasp with any cotton plant-related volatiles, nor does the parasitoid gain any experience with color or shape of a cotton plant. Therefore, the attraction of parasitoids to cotton plants is considered to be innate.

The attraction of the generalist *C. marginiventris* was greater to artificially damaged cotton plants than to undamaged plants. Artificially damaged and recently caterpillar-damaged cotton plants release green leafy volatiles and a

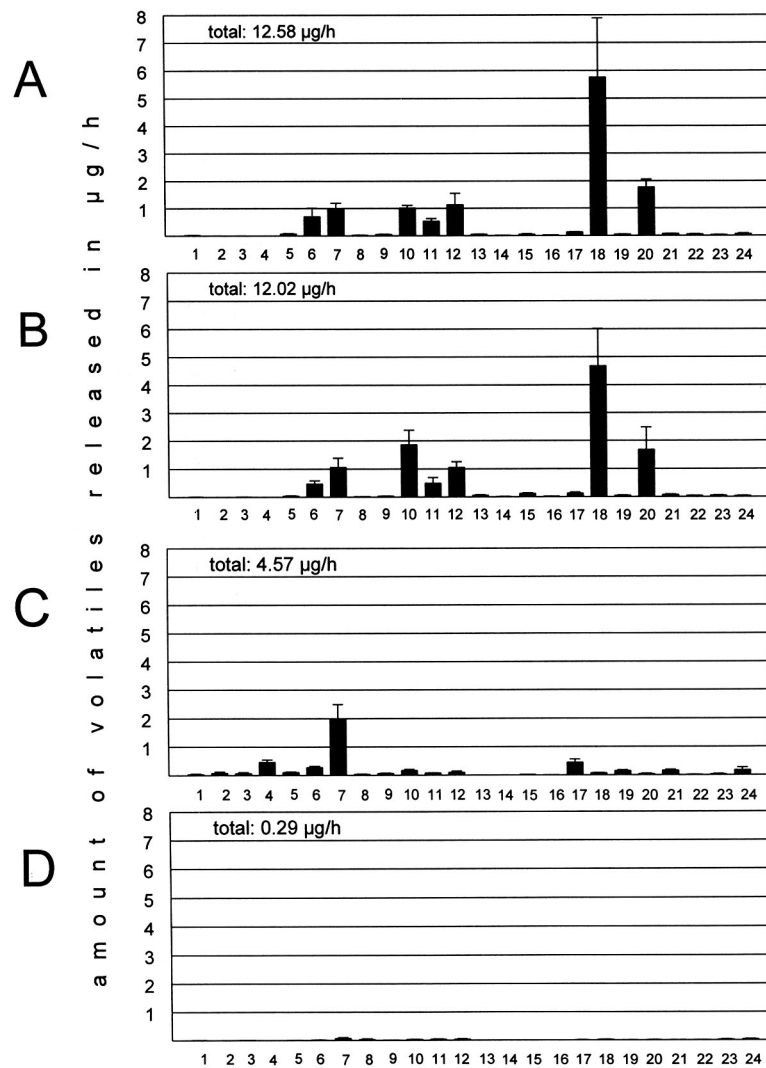


FIG. 1. Composition of volatile blends (mean over five replications with SEM) (A) systemically from undamaged leaves of a beet armyworm-damaged cotton plant after four days of feeding damage on the lower leaves (SYST-BAW), (B) systemically from undamaged leaves of a corn earworm-damaged cotton plant after four days of feeding damage on the lower leaves (SYST-CEW), (C) from artificially damaged cotton leaves (ART), and (D) an undamaged control plant (CTRL). Volatiles were collected from 11:00 to 12:00 hr for SYST-BAW and SYST-CEW, and from 12:00 to 15:00 hr for ART and CTRL plants. Volatiles were analyzed on a methyl silicone capillary column

number of constitutive mono- and sesquiterpenes that are different from the acyclic terpenoids that are released after several hours of herbivore damage (Loughrin et al., 1994; McCall et al., 1994). While the generalist *C. marginiventris* was increasingly attracted to plants that released those constitutive compounds, we did not observe an increase in the total number of completed flights for the specialist *M. croceipes* compared to the number of completed flights to control plants. A generalist like *C. marginiventris* can parasitize several different lepidoptera species that attack the same plant. Consequently, green leafy volatiles and constitutive compounds released from recently damaged plants may provide a useful cue for the location of a leaf-feeding host by the generalist. The specialist *M. croceipes* attacks only a limited number of lepidoptera larvae, one of them CEW, which prefers feeding on cotton squares and bolls. CEW-damaged cotton squares (Röse and Tumlinson, unpublished data) and bolls (Turlings et al., 1993a) release only small amounts of green leafy volatiles. Therefore, the chances of *M. croceipes* encountering a host on a plant that releases only green leafy volatiles and constitutive compounds are relatively small. This may explain the overall lower attraction to those compounds released from artificially damaged plants in our experiment.

Compared to the total number of flights completed by *M. croceipes* and *C. marginiventris* to artificially damaged plants, the level of response of the wasps to inducible volatiles systemically released in response to herbivore feeding was higher. *M. croceipes* and *C. marginiventris* responded strongly to cotton plants that released large amounts of inducible compounds systemically and preferred those plants over undamaged control plants. Because the wasps were again only allowed to antennate and sting a diet-fed host larva and did not have any previous experience with cotton volatiles, the attraction of the generalist and the specialist parasitoid species to systemically released cotton volatiles and the clear preference for those plants when compared to undamaged control plants appears to be innate. The innate preference of naive parasitoids of both species for plants that released inducible volatile compounds is high, whereas artificially or recently damaged plants that released constitutive compounds were less attractive to the wasps (Figure 2). Since inducible compounds are released in significant amounts

and the amount of volatiles adjusted to the amount released per hour. Compound names: 1, (Z)-3-hexenal; 2, (E)-2-hexenal; 3, (Z)-3-hexenol; 4, α -pinene; 5, β -pinene; 6, myrcene; 7, (Z)-3-hexenyl acetate; 8, hexenyl acetate; 9, limonene; 10, (E)- β -ocimene; 11, linalool; 12, (E)-4,8-dimethyl-1,3,7-nonatriene; 13, (Z)-3-hexenyl butyrate; 14, (E)-2-hexenyl butyrate; 15, indole; 16, (Z)-jasmonone; 17, β -caryophyllene; 18, (E)- β -farnesene; 19, α -humulene; 20, (E,E)- α -farnesene; 21, unknown sesquiterpene hydrocarbon; 22, nerolidol; 23, (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene; 24, unknown sesquiterpene.

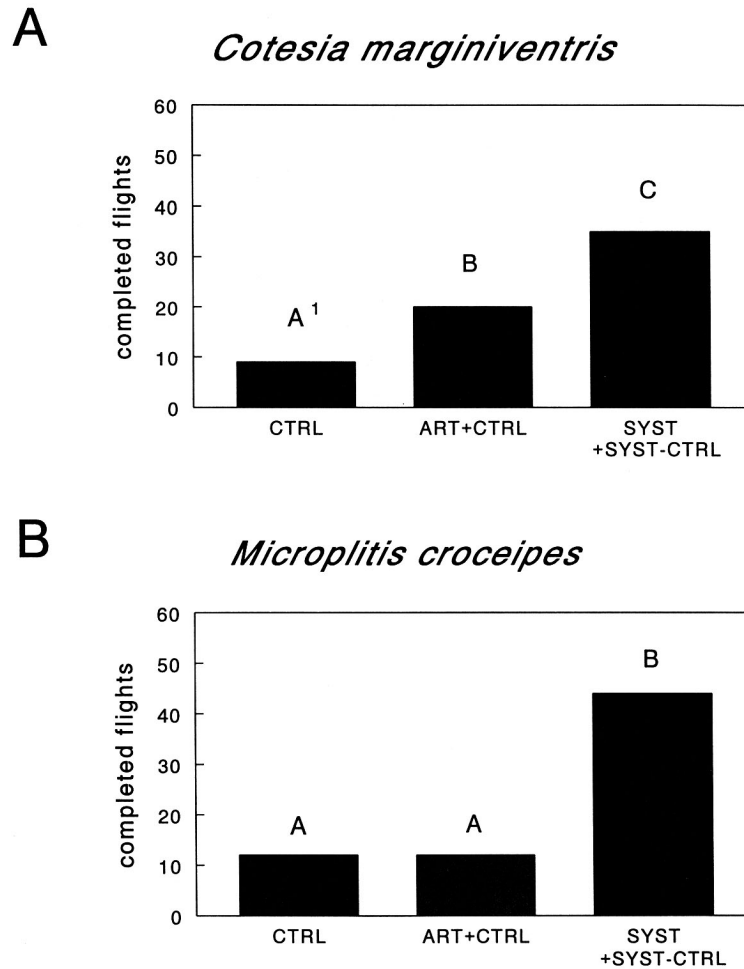


FIG. 2. Comparison of total number of wasps that completed flights to undamaged cotton plants (CTRL), to artificially damaged plants and undamaged plants (ART + CTRL), and to cotton plants that released inducible compounds systemically after feeding damage of beet armyworms on the lower leaves and to undamaged plants, both with the lower leaves removed from the plant (SYST + SYST - CTRL) (A) of the generalist *C. marginiventris* and (B) of the specialist *M. croceipes*. (¹Fischer's exact test was used to compare the significance of differences in the total number of completed flights to each treatment combination for each wasp species. The behavior was investigated on five different days with a total of $N = 60$ wasps per treatment. Different capital letters above bars indicate significant differences ($P \leq 0.05$).

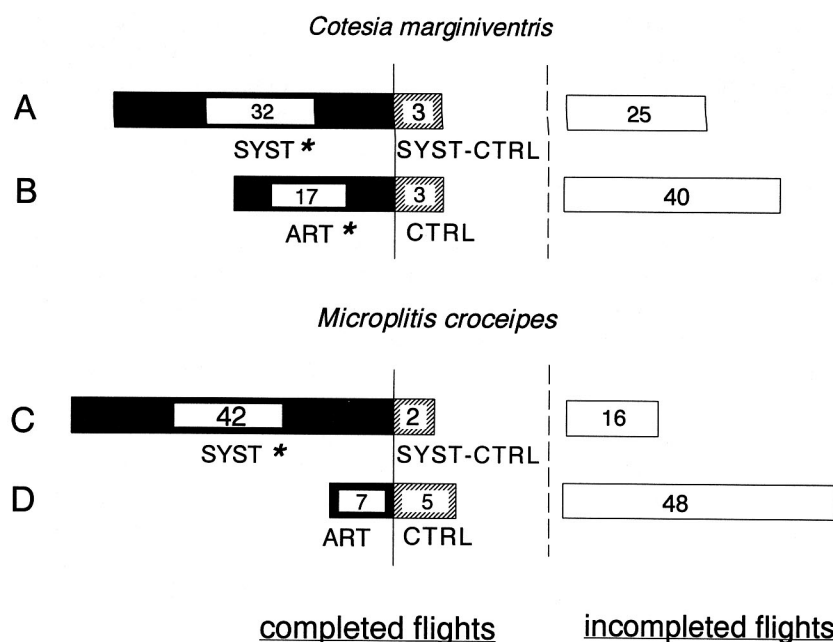


FIG. 3. Flight response of the generalist *C. marginiventris* and the specialist parasitoid *M. croceipes* in two-choice experiments to: (A, C) volatiles released systemically from undamaged leaves of a cotton plant damaged by beet armyworm larvae on the lower leaves (SYST) compared to an undamaged control plant (SYST – CTRL) both with the lower leaves removed; and (B, D) artificially damaged cotton leaves (ART) compared to an undamaged control plant (CTRL). The shaded bars indicate the numbers of wasps that landed on each source, and the open bars show those that did not land on either plant, for each test. Asterisks indicate significant differences in preferences within each pair of odors (Fischer's exact test, $P \leq 0.05$, $N = 60$).

in response to herbivore damage, they are a reliable indication of an herbivore-damaged plant.

Behavioral studies indicated previously that undamaged parts of cassava plants partly damaged by mealybugs were attractive to parasitoids (Nadel and van Alphen, 1987). Furthermore, undamaged parts of spider mite-infested lima beans were attractive to predators (Dicke et al., 1990a,b; Dicke and Dijkman, 1992). Corn seedlings that were partly damaged with a razor blade with caterpillar oral secretion rubbed over the damaged surface were attractive for the parasitoid *C. marginiventris* (Turlings and Tumlinson, 1992). The compounds released systemically by those corn seedlings, in amounts significantly larger than from control seedlings, were (Z)-3-hexenyl acetate and the terpenoids lin-

alool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. All of these previously reported compounds [(*Z*)-3-hexenyl acetate, (*E*)- β -ocimene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene] and in addition (*E*,*E*)- α -farnesene and (*E*)- β -farnesene were released from undamaged leaves of caterpillar-damaged cotton plants (Röse et al., 1996) and were also systemically released from cotton plants immediately prior to their use in the flight tunnel for our experiments. It appears that those compounds are not only released from several plant species in response to herbivory, but that those compounds also serve as attractants for a variety of predaceous and parasitic insects. However, since we could only collect volatiles from plants immediately prior to their use in the flight tunnel, but not during the flight-tunnel experiment itself, we can only assume that *M. croceipes* and *C. marginiventris* were responding to those systemically released volatiles.

In our experiments, *M. croceipes* were attracted to plants that released volatiles systemically in response to feeding damage of a nonhost (BAW) on the lower leaves. Our gas chromatographic analysis of systemically released volatiles in response to host (CEW) feeding damage on the lower leaves showed that there were no qualitatively and quantitatively significant differences between systemically released volatiles in response to BAW or CEW feeding on the lower leaves. In addition, some of the inducible compounds released systemically are known to be released from CEW-damaged cotton bolls [(*Z*)-3-hexenyl acetate, (*E*)- β -ocimene, and (3*E*)-4,8-dimethyl-1,3,7-nonatriene] (Turlings et al., 1993a). The similarities of herbivore inducible compounds systemically released after feeding damage of different herbivore species and also some similarities of volatile compounds released from different feeding sites on the plant may explain the attraction of *M. croceipes* that are naive with regard to cotton volatiles to inducible compounds systemically released after nonhost feeding damage. These findings are in accordance with results obtained from corn plants (Turlings et al., 1993b). When the cut stems of corn seedlings were placed in oral secretion of different lepidopteran species, those corn plants released the same compounds. The parasitoids *C. marginiventris* and *M. croceipes* were attracted to corn plants placed in BAW oral secretion. As in our study, *M. croceipes* were attracted to volatiles systemically released in response to a nonhost. However, with increasing experience of the plant-host complex, small ratio differences in volatile blends may become more important to the parasitoid. Then, wasps may learn to distinguish even between small differences in the blend (Turlings et al., 1993b), whereas the parasitoids in our experiment were naive with regard to cotton plant volatiles.

The specificity of the signal to herbivore damage, but not to the attacking herbivore species, can still be effective, as we previously discussed (Turlings et al., 1995). From the standpoint of the plant, it is not necessarily a disadvan-

tage to attract parasitoids or predators to unsuitable herbivores, as long as the natural enemies of the herbivores are attracted also. Inducible compounds that are systemically released in response to herbivore damage may easily be detected by parasitoids from a distance since those compounds are released in large amounts throughout the entire plant (Röse et al., 1996). This may benefit the herbivore damaged plant by increasing its apparency to beneficial insects. In cotton, these inducible compounds are synthesized *de novo* (Paré and Tumlinson, 1997b). Plant varieties that can respond quickly to herbivore damage by synthesizing and releasing large amounts of inducible compounds may attract beneficial insects faster and thereby minimize herbivore damage. However, from the standpoint of the parasitoid, obviously more specific cues are necessary for the successful location of a host larva. Parasitoids may use nonspecific plant volatiles as a long-range cue to lead them to the general area where they are likely to find a herbivore feeding on a plant. Once they have located such an area, wasps may rely on more specific cues such as host frass (Elzen et al., 1987; Eller et al., 1988; Turlings et al., 1991b; Steinberg et al., 1993; Röse et al., 1997; Cortesero et al., 1997).

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